Specific action mediated by spinal HMGB1 receptors in the induction of central post-stroke pain

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(Background) We have previously shown that spinal high-mobility group box-1 (HMGB1) plays an important role in the induction of central post-stroke pain (CPSP). It has been also reported that HMGB1 exacerbates inflammation and pain through toll-like receptor 4 (TLR4) or receptor for advanced end products (RAGE). Furthermore, we have suggested that HMGB1 regulates activation of glial cells and nitric oxide synthetase (NOS) involved in pain through TLR4 and RAGE. In this study, we investigated whether the interaction between spinal glial cells and HMGB1 signaling, including its receptors TLR4 or RAGE, is directly involved in the induction of CPSP.

(Methods) Bilateral carotid arteries of male ddY mice (5 weeks old) were occluded for 30 min (BCAO). Mechanical allodynia was evaluated by a von Frey filament test on day 3 after BCAO. On day 3 after BCAO, anti-HMGB1 monoclonal antibody (mAb) (20 μ g/mouse), lipopolysaccharides from *Rhodobacter sphaeroides* (LPS-RS, a TLR4 antagonist; 5 and 10 μ g/mouse), low-molecular-weight heparin (LMWH, a RAGE antagonist; 20, 40, and 70 μ g/mouse) and N^G-nitro-L-arginine methyl ester (L-NAME, a nonselective nitric oxide synthetase (NOS) inhibitor; 100 and 300 μ g/mouse) were intrathecally (i.t.) inject. Activation of glial cells and NOS activity on day 3 after BCAO were measured using immunostaining and colorimetric assay, respectively.

(Result) Spinal HMGB1 expression increased, and spinal microglia and astrocytes were also activated on day 3 after BCAO. The expression of HMGB1 colocalized with neurons, but not microglia and astrocytes, after BCAO. Intrathecal injection of LPS-RS and LMWH significantly blocked mechanical allodynia on day 3 after BCAO. BCAO-induced activation of spinal microglia and astrocyte were suppressed by i.t. injection of anti-HMGB1 mAb and LPS-RS, but not LMWH. In addition, i.t. injection of L-NAME significantly blocked mechanical allodynia on day 3 after BCAO and i.t. injection of anti-HMGB1 mAb, LPS-RS and LMWH significantly inhibited the increase of NOS activity in the spinal cord on day 3 after BCAO.

(Conclusion) These results show that spinal HMGB1/TLR4/glial cells/NOS and HMGB1/RAGE/NOS system are directly involved in the induction of CPSP.